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File: USPT

Aug 29, 2000

US-PAT-NO: [6110693](#)

DOCUMENT-IDENTIFIER: US 6110693 A

TITLE: Methods of assaying receptor activity and constructs useful in such methods

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

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US-CL-CURRENT: [435/7.2](#); [435/69.1](#), [435/7.1](#), [530/350](#), [536/23.4](#)

CLAIMS:

What is claimed is:

1. A method of detecting G protein coupled receptor (GPCR) pathway activity in a cell expressing at least one GPCR and containing .beta.-arrestin protein conjugated to an optically detectable molecule, said method comprising detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell, wherein said translocation of the detectable molecule indicates activation of the GPCR pathway;

and wherein said detecting step comprises (i) detecting an increase in said detectable molecule at said membrane edge; (ii) detecting a decrease in said detectable molecule in said cytosol; or (iii) detecting both an increase in said detectable molecule at said membrane edge and detecting a decrease in said detectable molecule in said cytosol.

2. A method according to claim 1 wherein said detection is of an increase in the detectable signal at the membrane edge of the cell over time.

3. A method according to claim 1 wherein said detection is of a decrease in the detectable signal in the cytosol of the cell over time.

4. A method according to claim 1 wherein said translocation is detected by comparing the distribution of the detectable signal in a test cell to the distribution of the detectable signal in a control cell.

5. A method according to claim 1 wherein said detection of the detectable signal occurs over time.

6. A method according to claim 1 wherein said translocation is detected by comparing the distribution of the detectable signal in a test cell to a pre-established standard.
7. A method according to claim 1 wherein said detectable molecule is photochemically detectable.
8. A method according to claim 1 wherein said detectable molecule is biochemically detectable.
9. A method according to claim 1 wherein said detectable molecule is immunochemically detectable.
10. A method according to claim 1 wherein said detectable molecule is spectroscopically detectable.
11. A method according to claim 1 wherein said cell is a mammalian cell.
12. A method according to claim 1, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.
13. A method according to claim 1 wherein the cell expresses a GPCR whose function is known.
14. A method according to claim 1 wherein the cell expresses a GPCR whose function is unknown.
15. A method according to claim 1 wherein the cell expresses an odorant GPCR.
16. A method according to claim 1, wherein the cell expresses a .beta.-adrenergic GPCR.
17. A method according to claim 1, wherein the cell endogenously expresses a GPCR.
18. A method according to claim 1, wherein the cell has been transformed to express a GPCR not endogenously expressed by such a cell.
19. A method according to claim 1, wherein the cells are deposited on a substrate prior to detecting translocation of the detectable molecule from the cytosol to the membrane edge.
20. A method according to claim 1 wherein said cell is contained in a tissue.
21. A method according to claim 1 wherein said cell is contained in an organ.
22. A method according to claim 1 wherein the cell expresses a taste GPCR.
23. A method according to claim 1 wherein the cell is an insect cell.
24. A method according to claim 1, wherein said detecting step comprises detecting an increase in said detectable molecule at said membrane edge.

25. A method according to claim 1, wherein said detecting step comprises detecting a decrease in said detectable molecule in said cytosol.

26. A method according to claim 1, wherein said detecting step comprises detecting both an increase in said detectable molecule at said membrane edge and detecting a decrease in said detectable molecule in said cytosol.

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L3: Entry 1 of 1

File: USPT

Apr 6, 1999

US-PAT-NO: [5891646](#)

DOCUMENT-IDENTIFIER: US 5891646 A

TITLE: Methods of assaying receptor activity and constructs useful in such methods

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION:

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Zhang; Jie	Durham	NC		

US-CL-CURRENT: [435/7.2](#); [435/69.1](#), [435/7.1](#), [530/350](#), [536/23.4](#)

CLAIMS:

That which is claimed:

1. A method of assessing the effect of a test condition on G protein coupled receptor (GPCR) pathway activity, comprising:

- a) providing a cell that expresses a GPCR, and that contains a conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;
- b) exposing the cell to an agonist for said GPCR under said test condition;
- c) detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell; and then
- d) comparing any translocation detected in step (c) to that which occurs in the absence of said test condition;

wherein increased translocation of the detectable molecule in the cell compared to that which occurs in the absence of said test condition indicates increased GPCR activation compared to activation that occurs in the absence of said test condition, and decreased translocation of the detectable molecule in the cell compared to that which occurs in the absence of said test condition indicates decreased GPCR compared to activation that occurs in the absence of said test condition.

2. A method according to claim 1, wherein the test condition is the presence in the cell of a kinase.

3. A method according to claim 1, wherein the test condition is the presence in the cell of a G-protein.

4. A method according to claim 1, wherein the test condition is the exposure of the cell to a compound selected from GPCR agonists and GPCR antagonists.

5. A method according to claim 1 wherein the test condition is co-expression in the cell of a second receptor.

6. A method for screening a .beta.-arrestin protein or fragment thereof for the ability to bind to phosphorylated GPCRs, comprising:

a) providing a cell that:

i) expresses at least one GPCR; and

ii) contains a conjugate comprising a test .beta.-arrestin protein and an optically detectable molecule;

b) exposing the cell to an agonist for said at least one GPCR; and then

c) detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell;

wherein translocation of the detectable molecule in the cell indicates .beta.-arrestin protein binding to the phosphorylated GPCR.

7. A method for screening a test compound for G protein coupled receptor (GPCR) agonist activity, comprising:

a) providing a cell expressing a GPCR and containing a conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;

b) exposing the cell to a test compound; and then

c) detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell;

wherein movement of the detectable molecule from the cytosol to the membrane edge of the cell after exposure of the cell to the test compound indicates GPCR agonist activity of the test compound.

8. A method according to claim 7 wherein the cell expresses a GPCR whose function is known.

9. A method according to claim 7 wherein the cell expresses a GPCR whose function is unknown.

10. A method according to claim 7 wherein the cell expresses an odorant GPCR.

11. A method according to claim 7 wherein the cell expresses a .beta.-adrenergic GPCR.

12. A method according to claim 7 wherein the detectable molecule is Green

Fluorescent Protein.

13. A method according to claim 7 wherein the cell is a mammalian cell.

14. A method according to claim 7, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

15. A method according to claim 7, wherein the cell endogenously expresses a GPCR.

16. A method according to claim 7, wherein the cell has been transformed to express a GPCR not endogenously expressed by such a cell.

17. A method according to claim 7, where the test compound is in aqueous solution.

18. A method according to claim 7, where the cells are deposited on a substrate prior to said detecting step.

19. A method of screening a sample solution for the presence of an agonist to a G protein coupled receptor (GPCR), comprising:

a) providing a cell expressing a GPCR and containing a conjugate, the conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;

b) exposing the cell to a sample solution; and then

c) detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell;

wherein movement of the detectable molecule from the cytosol to the membrane edge of the cell after exposure of the cell to the sample solution indicates the sample solution contains an agonist for a GPCR expressed in the cell.

20. A method according to claim 19, wherein the cell expresses a GPCR whose function is known.

21. A method according to claim 19, wherein the cell expresses a GPCR whose function is unknown.

22. A method according to claim 19 wherein the cell expresses an odorant GPCR.

23. A method according to claim 19 wherein the cell expresses a .beta.-adrenergic GPCR.

24. A method according to claim 19 wherein the detectable molecule is Green Fluorescent Protein.

25. A method according to claim 19 wherein the cell is a mammalian cell.

26. A method according to claim 19, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

27. A method of screening a test compound for G protein coupled receptor (GPCR) antagonist activity, comprising:

- a) providing a cell expressing a GPCR, and containing a conjugate, the conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;
- b) exposing the cell to a test compound;
- c) exposing the cell to an agonist for said GPCR; and
- d) detecting translocation of the detectable molecule from the cytosol to the membrane edge of the cell;

where exposure to the agonist occurs at the same time as, or subsequent to, exposure to the test compound, and wherein movement of the detectable molecule from the cytosol to the membrane edge of the cell after exposure of the cell to the test compound indicates that the test compound is not an antagonist for said GPCR.

28. A method according to claim 27 wherein the cell expresses a GPCR whose function is unknown.

29. A method according to claim 27 wherein the cell expresses an odorant GPCR.

30. A method according to claim 27 wherein the cell expresses a .beta.-adrenergic GPCR.

31. A method according to claim 27 wherein the detectable molecule is Green Fluorescent Protein.

32. A method according to claim 27 wherein the cell is a mammalian cell.

33. A method according to claim 27, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells, and animal cells.

34. A method according to claim 27, where the cells are deposited on a substrate prior to said detecting step.

35. A method for screening a test compound for G protein coupled receptor (GPCR) antagonist activity; comprising:

- a) providing a cell expressing a GPCR and containing a conjugate, the conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;
- b) exposing the cell to a GPCR agonist so that translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell occurs;
- c) exposing the cell to a test compound; and
- d) detecting translocation of the detectable molecule from the membrane edge of the cell to the cytosol of the cell;

where exposure to the agonist occurs prior to exposure to the test compound, and wherein movement of the detectable molecule from the membrane edge of the cell to the cytosol after exposure of the cell to the test compound indicates that the test compound has a GPCR antagonist activity.

36. A method according to claim 35 wherein the detectable molecule is Green Fluorescent Protein.

37. A method according to claim 35 wherein the cell is a mammalian cell.

38. A method according to claim 35, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

39. A method according to claim 35, where the test compound is in aqueous solution.

40. A method according to claim 35, where the cells are deposited on a substrate prior to said detecting step.

41. A method of screening a cell for the presence of a G protein coupled receptor (GPCR) responsive to a GPCR agonist, comprising:

a) providing a cell, said cell containing a conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;

b) exposing the cell to a GPCR agonist; and

c) detecting translocation of the detectable molecule from the cytosol to the membrane edge of the cell;

wherein movement of the detectable molecule from the cytosol to the membrane edge of the cell after exposure of the cell to the GPCR agonist indicates that the cell contain a GPCR responsive to said agonist.

42. A method according to claim 41 wherein the detectable molecule is Green Fluorescent Protein.

43. A method of screening a plurality of cells for those cells which contain a G protein coupled receptor (GPCR) responsive to a GPCR agonist, comprising:

a) providing a plurality of cells, said cells containing a conjugate comprising a .beta.-arrestin protein and an optically detectable molecule;

b) exposing the cells to a GPCR agonist; and

c) detecting those cells in which the detectable molecule is translocated from the cytosol of the cell to the membrane edge of the cell;

wherein movement of the detectable molecule from the cytosol to the membrane edge of a cell after exposure to the GPCR agonist indicates that the cell contains a GPCR for said GPCR agonist.

44. A method according to claim 43 wherein the detectable molecule is Green

Fluorescent Protein.

45. A method according to claim 43 wherein the plurality of cells are contained in a tissue.

46. A method according to claim 43 wherein the plurality of cells are contained in an organ.

47. A method according to claim 43 wherein step (b) comprises exposing the cells to a plurality of GPCR agonists.

48. A substrate having deposited thereon a plurality of cells, said cells expressing at least one GPCR and containing a conjugate, the conjugate comprising a .beta.-arrestin protein and a detectable molecule selected from the group consisting of optically detectable molecules and immunologically detectable molecules.

49. A substrate according to claim 48, wherein the detectable molecule is Green Fluorescent Protein.

50. A substrate according to claim 48 wherein the cells are mammalian cells.

51. A substrate according to claim 48, wherein said plurality cells are selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

52. A substrate according to claim 48 wherein the cells express an odorant GPCR.

53. A substrate according to claim 48 wherein the cells express a .beta.-adrenergic GPCR.

54. A substrate according to claim 48, wherein the substrate is made of a material selected from glass, plastic, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer, and biocompatible polymer materials.

55. A method of detecting G protein coupled receptor (GPCR) pathway activity in a cell expressing at least one GPCR and containing .beta.-arrestin protein conjugated to an optically detectable molecule, said method comprising detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell, wherein said translocation of the detectable molecule indicates activation of the GPCR pathway.

56. A method according to claim 55 wherein said detectable molecule is Green Fluorescent Protein.

57. A method according to claim 55 wherein said cell is a mammalian cell.

58. A method according to claim 55, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

59. A method according to claim 55 wherein the cell expresses a GPCR whose function is known.

60. A method according to claim 55 wherein the cell expresses a GPCR whose

function is unknown.

61. A method according to claim 55 wherein the cell expresses an odorant GPCR.

62. A method according to claim 55, wherein the cell expresses a .beta.-adrenergic GPCR.

63. A method according to claim 55, wherein the cell endogenously expresses a GPCR.

64. A method according to claim 55, wherein the cell has been transformed to express a GPCR not endogenously expressed by such a cell.

65. A method according to claim 55, where the cells are deposited on a substrate prior to detecting translocation of the detectable molecule from the cytosol to the membrane edge.

66. A method according to claim 55 wherein said cell is contained in a tissue.

67. A method according to claim 55 wherein said cell is contained in an organ.

68. A method according to claim 1 wherein the cell expresses a taste GPCR.

69. A method according to claim 7 wherein the cell expresses a taste GPCR.

70. A method according to claim 17 wherein the cell expresses a taste GPCR.

71. A method according to claim 27 wherein the cell expresses a taste GPCR.

72. A method according to claim 35 wherein the cell expresses a taste GPCR.

73. A method according to claim 41 wherein the cell expresses a taste GPCR.

74. A method according to claim 55 wherein the cell expresses a taste GPCR.

75. A method according to claim 1 wherein the cell is an insect cell.

76. A method according to claim 7 wherein the cell is an insect cell.

77. A method according to claim 14 wherein the cell is an insect cell.

78. A method according to claim 27 wherein the cell is an insect cell.

79. A method according to claim 35 wherein the cell is an insect cell.

80. A method according to claim 41 wherein the cell is an insect cell.

81. A method according to claim 55 wherein the cell is an insect cell.

82. A method according to claim 43 wherein said plurality of cells are insect cells.

83. A substrate according to claim 48 wherein said plurality of cells are insect cells.

84. A substrate according to claim 48 wherein the cells express a taste GPCR.

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